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Temporal variation of hydrophobic organochlorine contaminants in the zebra mussel Dreissena polymorpha in relation to physiological and limnological factors.

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Temporal variation of hydrophobic organochlorine contaminants in the zebra mussel *Dreissena polymorpha* in relation to physiological and limnological factors.

By

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ABSTRACT

Zebra mussels (Dreissena polymorpha) have been recommended as biomonitors of metal and organic contaminants. Partitioning of hydrophobic contaminants into an organism depends on, among other factors, the lipid content of the organism. Mathematical models used to predict the fate of contaminants in the environment frequently assume that the lipid content within an organism remains constant over time, despite empirical evidence to the contrary. In this thesis, I assess the influence of temporal variation in lipid content and reproductive activity on contaminant dynamics in zebra mussels collected from western Lake Erie adjacent to the Fermi Nuclear Plant. Mussels spawned twice: first in June and again in August. Lipid content of mussels was maximal following the first spawning event and declined steadily thereafter. Principal Component Analysis separated physiological and limnological parameters into three factors: seasonal (date, lipid and temperature), reproductive (reproductive status) and limnological (Chlorophyll a, secchi depth and temperature). Multiple Regression Analyses revealed that seasonal, reproductive, and limnological factors were of equal importance. Contaminant concentrations were positively related to lipid content and reproductive status. However, the effect of spawning activity is unclear, at it did not directly affect lipid content or contaminant concentrations. These results suggest that other physiological or limnological factors may obscure the effect of spawning activity on lipid content and contaminant dynamics in zebra mussels.

Examination of the lipid composition revealed that polar lipids (61 % of total lipids) are dominant in zebra mussels. Concentrations of PCBs with log K_{ow} > 5.71 were more highly correlated with neutral lipid content than with total lipid content. This finding suggests that lipid normalization on a neutral lipid basis may be more appropriate than total lipid adjustment. Furthermore, compositional changes in lipid content may dramatically affect hydrophobic contaminant dynamics in mussels.

In memory of E.B. whose spirit remains with me, always.

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INTRODUCTION

Polychlorinated biphenyls (PCBs) are a group of polyhalogenated aromatic compounds that were first commercially synthesised in 1929. They were once widely used as heat transfer fluids, organic diluents, plasticizers, fire retardants, paint additives, and dielectric fluids for capacitors and transformers. However, the very chemical properties that make these compounds industrially useful, namely persistence, and resistance to breakdown by heat, hydrolysis, acids and bases, have also resulted in widespread environmental concerns (Safe 1990). As such, the manufacture, use and discharge of PCBs was restricted in 1972 in North America. This led to a significant global decline in PCB concentrations (Loganathan and Kannan 1991, 1994; Harrad et al. 1994; Gobas et al. 1995). However, PCBs are still considered a priority pollutant in the Great Lakes basin owing to their ubiquitous distribution, toxicity to aquatic organisms and long residence time. Indeed, PCBs may pose a greater environmental threat than even highly toxic dioxins due to their relatively high environmental concentrations and widespread distribution (Tanabe et al. 1987a).

Polychlorinated organic contaminants are typically present in extremely low concentrations in lake and river water, but some readily accumulate in biota. Biomonitors, defined as any organism or characteristic thereof used to assess environmental quality, have been successfully used to identify and quantify polychlorinated organic contaminants in aquatic environments. Because of their bioconcentration abilities, indicator organisms integrate both present and past exposure histories, thereby dampening short-term fluctuations in the environment (Phillips 1980). The use of biomonitors can complement and/or supplement traditional water and sediment monitoring programs and, perhaps most importantly, provide information on the *bioavailability* of contaminants (i.e. relative amounts of freely dissolved, non-sorbed contaminants available to bioaccumulate) (Rosenberg and Resh 1993). Biomonitors can

also reveal emission sources, define impact zones and serve as a feedback mechanism for assessing pollution control devices (Metcalfe and Charlton 1990).

Molluscs have many inherent characteristics that have resulted in their world-wide use as biomonitors (Najdek and Bazulic 1988; Claisse 1989; Ramesh et al. 1990; Ruangwises et al. 1994). Molluscs are in intimate contact with their local aquatic environment because they are sessile and filter large quantities of water. Their relative longevity fosters environmental equilibrium with even highly hydrophobic contaminants (see below). They are also of sufficient size for collection and analyses. Further, molluscs are known for having extremely low levels of microsomal mono-oxygenases (Walker 1987). These enzymes are capable of metabolizing most organic compounds and thereby complicating the relationship between contaminant concentrations in the organism and ambient water (Walker 1987).

In the past, native and introduced unionid molluscs were employed as biomonitors in 'Mussel Watch' programs on the Great Lakes (Kauss and Hamdy 1985; Pugsley et al. 1985; Muncaster et al. 1989; Russell and Gobas 1989). However, many unionid species have been virtually extirpated from some major North American lake and river systems, particularly Lake St. Clair and parts of Lake Erie, owing to zebra mussel (Dreissena polymorpha Pallas) fouling (Bogan 1993; Haag et al. 1993; Schloesser et al. 1996). Conversely, the North American distribution of the zebra mussel has expanded; the species now ranges from the St. Lawrence River to the southern Mississippi River at Baton Rouge, LA. The zebra mussel was first reported on the southern margin of Lake St. Clair in 1988, though the species probably established in the lake in 1986 (Hebert et al. 1989).

Zebra mussels have been used extensively in Europe as biomonitors of heavy metal and organochlorine contaminant exposure (Duursma et al. 1984; Kraak et al. 1991; Mersch et al. 1992; Giese and Krüger 1992). The mussel's utility as a biomonitor is a consequence of its life history. Because Dreissena is characterized by a benthic, sessile

and relatively long-lived (2-3 years) adult stage, it is useful for assessing spatial and temporal trends in water quality. Dreissenid mussels are filter feeders, preferentially straining particles between <1 to ~50 μm from the water column (Ten Winkel and Davids 1983; Sprung and Rose 1988; Cotner et al. 1995, MacIsaac and Rocha 1995; Silverman et al 1995). Mussel filtering rates vary as a function of mussel size, food concentration and temperature (Walz 1978a; MacIsaac 1996); filtering activities keep individuals in continuous contact with the local environment, thereby imparting enhanced sensitivity to the presence of temporally variable contaminants. Further, the mussel's extensive distribution in North America facilitates comparison between distant sites.

Owing to their hydrophobic nature, most organochlorine compounds readily partition into the lipid fraction of aquatic biota. The relatively high lipid content of Dreissena (9 to 15% of dry weight) makes it especially suitable to monitor trace organic contaminants (Mersch et al. 1992; Fisher et al. 1993; Bruner et al. 1994). By comparison, the European marine mussel Mytilus galloprovincialis and the fresh water mussel Elliptio complanata have lipid contents of 1.3 and 2.5% of dry weight, respectively (Solé et al. 1994; Muncaster et al. 1989). Zebra mussels bioaccumulate organic contaminants to a dry-mass concentration of approximately one order of magnitude higher than other bivalves (e.g. Lampsilis siliquoidea and Mytilus, Brieger and Hunter 1993; Fisher et al. 1993). Thus, trace amounts of bioavailable contaminants can be readily quantified in the tissue fraction of zebra mussels that would otherwise be undetectable in water samples.

The primary route of organic contaminant exposure and accumulation for organisms occupying lower trophic positions is the freely dissolved phase of water. Particulate organic matter, dissolved organic matter and food are thought to have little influence on contaminant uptake (Thomann 1981; Pruell et al. 1986; Tanabe et al. 1987b; Connolly and Pederson 1988). Since the 1980s, equilibrium partitioning has been the principal mechanism used to explain the accumulation of hydrophobic chemicals into lipid compartments of aquatic biota (Mackay 1982; Tanabe et al. 1987b; Hummel et al.

1989, 1990; Kannan et al. 1989; Secor et al. 1993). Equilibrium partitioning, a thermodynamic model, is based primarily on the natural tendency of hydrophobic compounds to partition out of the aqueous phase into the energetically favourable organic phase of the system. Accumulation occurs through the passive diffusion of contaminants between water and an organism *via* the gills. Transport across the gills continues until the fugacity (f) of the organism and that of its environment are equal. Fugacity of a phase is estimated as:

$$f = C/Z \qquad \text{eq. 1}$$

where C is the concentration of the chemical and Z is the capacity of the phase to accommodate the chemical. Therefore at equilibrium,

$$F_b/F_w = C_bZ_w/C_wZ_b = 1. \qquad \text{eq. 2}$$

where subscripts b and w refer to biota and water, respectively.

The octanol-water partition coefficient (K_{ow}) approximates the relationship between biota and water capacities such that:

$$K_{ow} \sim Z_b/Z_w. \qquad \text{eq. 3}$$

Also,

$$K_{ow} \sim K_{oc} \sim Z_{oc}/Z_w \qquad \text{eq. 4}$$

where K_{oc} is organic carbon - water partition coefficient and Z_{oc} is fugacity capacity of organic carbon. Therefore,

$$Z_b = Z_{oc} \qquad \text{eq. 5}$$

Thus, contaminants theoretically partition equally among organic carbon phases regardless of the origin of the carbon. This has only been demonstrated for abiotic organic carbon phases (i.e. soils, sediment) and possibly, microrganisms (Karickhoff 1981, 1984; DiToro 1985). In biotic phases, contaminants differentially partition into the hydrophobic lipid component. Fugacity capacity of biota (Z_b; eq. 3) is represented by biotic lipid content and contaminant concentration of biota (C_b; eq. 2) is lipid-adjusted.

Substituting eq. 3 into eq. 2:

$$F_b/F_w \sim C_b/C_w K_{ow} \sim 1; \text{ therefore,} \qquad \text{eq. 6}$$

$$K_{ow} \sim C_b/C_w; \text{ but,} \qquad \text{eq. 7}$$

$$BCF_L = C_b/C_w \qquad \text{eq. 8}$$

where, BCF_L is the lipid-adjusted bioconcentration factor.

Thus K_{ow} is thought to be a good predictor of bioconcentration abilities, where BCF increases with increasing K_{ow}. This association can in turn be used to estimate uptake and elimination kinetics of contaminants. Implicit in these models, however, are several assumptions including: that the system is in equilibrium, that octanol is a good surrogate for lipid, that lipid content remains constant over time and that lipid is a homogeneous phase (Connell 1988). However, the lipid pool in bivalves fluctuates seasonally. For example, the lipid content of the mantle (site of gonad development) in Mytilus increased 1.5 to 2-fold during gametogenesis, whereas the lipid content of the

rest of the soft tissue did not change (Hummel et al. 1989). Bruner et al. (1994) demonstrated marked seasonal variation in lipid concentrations of zebra mussels in Lake Erie. Lipid concentrations were typically highest during June and July (15-20% dry-mass) and lower during spring and fall (7-10% dry-mass) (Bruner et al. 1994; Sprung 1995). Thus, temporal variation of the lipid pool due to gametogenesis and spawning activity could affect accumulation and release of organochlorine contaminants, and may therefore require consideration in kinetic-based models for biomonitoring.

Considering the reproductive effort in Dreissena is high relative to other molluscs (up to 45% annual production: Sprung 1995), reproductive activities could potentially impact lipid and contaminant dynamics. For instance, spawning may cause a substantial decrease in overall contaminant body burden if the contaminants are stored in the high-lipid egg fraction. Reproductive status may therefore be an important consideration when employing mussels as biomonitors.

Furthermore, lipid composition is known to differ between species as well as between tissues of a single organism. The relative proportions of lipid constituents, of various hydrophobicity, can also fluctuate seasonally (Lubert et al. 1985; Hummel et al. 1990; Delbeke et al. 1995). As such, a second dimension of equilibrium partitioning may occur within an individual.

The objective of this thesis is to assess temporal variability in organic contaminant burdens in zebra mussels in relation to variation in environmental and reproductive factors. Specifically, by monitoring seasonal variation in organochlorine concentrations in different zebra mussel tissues from a contaminated site in western Lake Erie, and by conducting concurrent analyses of relevant environmental factors and mussel reproductive status and lipid content and composition, I assess the role of reproductive maturity and spawning activity in altering contaminant body burdens. I hypothesize that as mussels sexually mature, lipid content should increase proportionately, and that spawning should result in a decrease in lipid as gametes are shed. If equilibrium

partitioning is an appropriate model and lipid is the site of contaminant accumulation, then changes in contaminant body burdens (wet-mass) should parallel lipid fluctuations whereas lipid-adjusted contaminant concentrations should remain constant over time.

METHODS

Study Site

Zebra mussels were collected from a chemically contaminated site in western Lake Erie adjacent to the Fermi Nuclear Plant in southeastern Michigan (Figure 1) and is ~7 m deep and covered with a dolomite bedrock bottom (pers. obs.). This site is directly downstream from the Detroit River outflow. The Detroit River receives a plethora of municipal and industrial (e.g. automotive, pharmaceutical) waste discharges primarily from the Michigan shoreline between Detroit and Trenton (Hamdy and Post 1985; Oliver and Bourbonniere 1985; Furlong et al. 1988). These discharges are contaminated with a variety of complex hydrophobic organic compounds and heavy metals. Consequently, the river is the principal source of biologically available PCBs and other contaminants entering the western basin of Lake Erie (Kauss and Hamdy 1985; Oliver and Bourbonniere 1985; Suns et al. 1985). PCBs enter Lake Erie principally *via* translocation of contaminated sediment. Oliver and Bourbonniere (1985) estimated that the Detroit River contributes 900-1000 kg annually of sediment-associated PCBs to the western basin of Lake Erie. Total PCB concentration in sediment near the study site averages 365 ng g^{-1} (Furlong et al. 1988; Hamdy and Post 1985; Kaiser et al. 1985; Oliver and Bourbonniere 1985). These PCBs are made available to biota for bioaccumulation through sediment re-suspension (Fallon and Horvath 1985).

Reproduction in Zebra Mussels

In the western basin of Lake Erie, gametogenesis begins in early spring or perhaps late winter. Gonads appear by the first week of May and reach maturity by August (Mackie 1991). Male and female mussels show similar patterns of sexual maturation between December and late August (Garton and Haag 1993). In 1988 synchronous

spawning occurred once in late August (Haag and Garton 1992). Gametogenesis typically ceases in September (Mackie 1991).

Sample Collections

Mussels were gathered every 10 to 14 days between 12 May and 04 October 1995, save one period of 17 days in July due to stormy weather. A total of 14 collections were made (Table 1). On each date, three replicates of several hundred mussels each were obtained by towing a benthic D-net trawl along the lake bottom. Three consecutive tows (each being one replicate) were taken running south to north, parallel to the Michigan shore. The starting point (N41°57.886 and W83°14.114) for the first tow was located via a Global Positioning System 45 (GARMIN Corp.) and each tow was approximately 300 m in length. After collection, mussels were thoroughly rinsed in lake water and placed in coolers containing lake water for transport to the laboratory.

Bottom temperature, secchi disk depth, and water depth were measured on each sampling date. Water samples for chlorophyll a analysis were taken with a modified Schindler-Patalis trap lowered to approximately 0.5 m above the lake bottom. Water was emptied into 1 L amber bottles. Bulk surface water samples were collected in 20 L carboys for use in laboratory aquaria.

Laboratory Protocol

Upon return to the laboratory, a random sub-sample of mussels (15 - 20 mm in length) from each replicate was immediately wrapped in hexane-rinsed foil and frozen at -20°C until lipid and contaminant analysis could be performed. Remaining mussels were transferred to three 40 L aerated glass aquaria containing lake water collected and glass-fibre filtered (Whatman 0.7 µm filter) that day. Mussels were kept in a cold room (12-14°C) with replicates in separate aquaria.

To isolate the effect of spawning activity on total contaminant body burdens, animals were induced to spawn in the laboratory. Following each mussel collection, a small trial was first conducted to ensure the mussels were competent to spawn. For this procedure, twenty mussels, 15-20 mm in length, were placed individually in 20 mL scintillation vials with 9 mL of filtered lake water and acclimated for 1 hr at 23-25°C. To induce spawning, 1 mL of 10^{-2} M serotonin (creatine sulphate salt of 5-hydroxytryptamine; Sigma Co.) was added to each vial to yield a final concentration of 10^{-3} M (Ram et al. 1993). A maximum of 4 hr was allotted for a response (Ram et al. 1993). If greater than 50% of the mussels spawned during the trial, 500 mussels from each replicate (20 June and 20 July) or 100 mussels from each replicate (11 August) were similarly induced to spawn. After spawning, female and male mussels were kept separate, wrapped in hexane rinsed foil and frozen at -20°C. A few (3-5) spawned mussels were dissected and examined microscopically to determine if spawning had been complete. In most cases spawning was only partial, though no effort was made to quantify its extent.

On 20 June and 20 July 1995 attempts were made to collect the egg fraction from spawned female mussels for direct contaminant analysis. Centrifugation, sedimentation and filtration techniques were attempted but were unsuccessful largely due to the time involved (approx. 6 hr from spawning) in preparing and collecting a sufficient mass of eggs for organic contaminant analysis; unfertilized eggs degenerated rapidly despite conducting the work in a cold room at 12 -14°C to help limit this process. On 11 August only enough mussels for whole animal (i.e. not the egg fraction) contaminant analysis were spawned.

On each date, thirty mussels from each replicate were microscopically examined to determine status of reproductive maturity (Fisher et al. 1993). Female mussels were scored on a scale of 0 to 4: 0 = spent; 1 = immature with males and females indistinguishable; 2 = immature with sexes differentiated; 3 = less than 50% eggs mature;

4 = greater than 50% of eggs mature. Male mussels were not scored. Whenever possible, the diameter of ten randomly-selected eggs from each female were measured with an ocular micrometer.

Water samples for chlorophyll a analysis were filtered onto acetate filters (0.8 µm; Gelman Sciences), which were then dissolved in 20 mL of 90% acetone neutralized with magnesium carbonate. Samples were stored in the dark overnight at 5°C. Samples were centrifuged and absorbance measured with a narrow band spectrophotometer according to Lind (1979).

Contaminant Analysis

Replicates from 11 of the 14 collection dates (Table 1) and laboratory-spawned male and females mussels were haphazardly selected for organic chemical analysis. All organic contaminant analyses were conducted in the Great Lakes Institute for Environmental Research organic chemistry laboratory at the University of Windsor, Windsor ON. Sample preparation and analysis techniques follow those of Lazar et al. (1992). Briefly, frozen mussels were shucked and soft tissue (1.5 to 4.5 g/replicate) was placed on hexane-rinsed aluminium weigh boats. Water from thawed tissue samples was drained and tissues were homogenized with 20 g of anhydrous sodium sulphate to a flowing powder. Ground tissue was poured into a glass column containing 20 g sodium sulphate soaked with 100 mL of a dichloromethane : hexane (1:1; v/v) solution. The samples were capped with 10 g of sodium sulphate. After 1 hr, the columns were slowly eluted and the extracts were collected in round bottom flasks, each containing 5 mL isooctane. The extracts were rotor-evaporated to 5 mL. For lipid determination, extracts were transferred to 50 mL graduated centrifuge tubes. Volumes were brought up to 25 mL with hexane. Two mL of each extract were removed and placed in pre-weighed 50 mL glass beakers. The solvent was evaporated and residues were dried at 105°C for 1 hr. Beakers were cooled to room temperature in a desiccator and re-weighed. The weight

difference is the weight of the lipid. Percent lipid was calculated from the initial wet weight of the mussels' tissues.

The remaining 23 mL of each extract were rotor-evaporated to approximately 5 mL and transferred to a second glass column containing 6 g of activated florosil and 50 mL of hexane. The column extracts were slowly eluted to round bottom flasks each containing 2 mL of isooctane. The mixtures were rotor-evaporated to approximately 1 mL and transferred to 2 mL volumetric flasks. Isooctane was added to bring volumes to 2 mL. A small amount of activated copper was added to capture sulphates which contaminate the sample. The sample was vortexed and refrigerated overnight. One mL of extract was transferred to a 2 mL glass vial and secured with a Teflon-lined lid suitable for gas chromatography.

Two µL of concentrated extract were injected using a splitless injection mode into a Hewlett Packard model 5890 Gas Chromatograph equipped with a 30 m x 0.25 mm x 0.25 mm DB-5 film thickness, ^{63}Ni electron capture detector, HP-7673A auto sampler and HP-3396 integrator. Injection temperature was 250°C, with He as the carrier gas (1 mL min^{-1} yielding a final velocity of 30 cm sec^{-1}). Oven temperature programme was as follows: initial temperature = 100°C; initial time = 1 min; rate = 10°C min^{-1} to 150°C, then 3°C min^{-1} to 275°C; final hold time = 5 min; equilibration time = 3 min. An extraction blank and 3 calibrated standard solutions provided by Canadian Wildlife Service Laboratories (Ottawa) were run with every six samples.

Lipid Class Analysis

To investigate temporal variation of different lipid classes and to compare contaminant concentrations normalized by different lipid classes, lipid content of mussels was analyzed using Iatroscan. Three replicates from collection dates representative of sexually immature, gravid, and spent mussels (Table 1 and 6) were selected for analysis. Total lipid was first extracted using the Bligh and Dyer (1959) technique modified by

Kates (1986). Briefly, 6 g of wet, previously frozen, zebra mussel tissues were homogenized twice: first with a chloroform : methanol (1:2; v/v) solution and second with a chloroform : methanol : 0.2 N HCl (2:1:0.8; v/v/v) solution. Samples were centrifuged (20 min at 3000 g) after each homogenization and the combined supernatants were diluted with 20 mL each of chloroform and water and centrifuged again. The upper methanol/water layer was discarded and the lower chloroform layer placed in a separatory funnel to complete the separation. The chloroform layer was withdrawn and neutralized with a drop of 0.2 N methanolic NH_4OH. Benzene was added to the extract to eliminate trace amounts of water during concentration with a rotor-evaporator. The residue was then dissolved in a small volume (10 mL) of a chloroform : methanol (2:1; v/v) solution. Three extraction blanks were randomized with the samples.

For dry weight of total lipid, 4 mL of the extract was pipetted into a pre-weighed 40 mL beaker. The solvents were evaporated under a stream of nitrogen and the remaining residue dried for 1 hour at 105°C. After cooling at room temperature in a desiccator, the beaker was re-weighed. The weight difference represents the mass of the lipid. Percent lipid was calculated on a wet-weight basis.

Of the remaining extract, 1 mL was placed in a 2 mL glass vial secured with a Teflon-lined cap and frozen at -20°C until lipid separation. Separation of the lipid classes was performed by Joann Cavaletto at the National Oceanic and Atmospheric Administration laboratory in Ann Arbor, MI using an Iatroscan equipped with a flame ionization detector. Methodology and procedure followed Parrish (1987). Iatroscan is similar to Thin Layer Chromatography (TLC) except that lipid extracts (15-20 mg) are spotted onto silica coated glass rods (chromarods) instead of traditional TLC plates. The lipids are separated by placing ("developing") the rods in progressively more polar solvent systems. After each incubation with solvents, the rods are placed in the Iatroscan. As the glass rods pass over the flame, the target lipids are burned off. The first scan detects the most non-polar lipid classes (mainly hydrocarbons and wax esters), the second

scan triglyceride, triacylglycerol, free fatty acids and free sterols, and the third scan acetone-mobile polar lipids and phospholipids.

Partitioning of Radio-labelled PCB

To verify differential partitioning of contaminants between somatic and gonadal zebra mussel tissues, gravid mussels were exposed to radiolabelled ^{14}C-PCB 153. Gravid mussels collected on 1 August were placed in 36 L glass aquaria (equipped with charcoal filters) containing pre-filtered (Whatman 0.7 µm glass-fibre filter) lake water collected from the sample site. The mussels were allowed to depurate for four weeks. During this time, mussels were fed a suspension of dry Chlorella every 2 -3 days (0.5 g Chlorella/g dry tissue mass/ L; Acta Pharmacal Co.). Condition index of the mussels was calculated weekly to ensure that good health of the mussels was maintained. Condition index was determined by harvesting 10 individuals (15-20 mm) and drying the shell and soft tissues separately at 70^{o}C for 48 hours. Condition index was calculated as soft tissue dry weight ÷ shell dry weight.

Two weeks prior to contaminant exposure, mussels (15-20 mm) were scrubbed clean and allowed to re-attach to glass microscope slides placed on the aquaria floor. Early attachment to slides reduced stress when mussels were later transferred to experimental tanks. Mussels were not fed for 2 days prior to, or during, contaminant exposure.

Ten days prior to the designated exposure period, the experimental aquaria were set up to allow adequate time for the systems to equilibrate. Conditions of the experiment (e.g. temperature, exposure time) were optimized according to findings from Fisher et al. (1993), P. Landrum (pers. comm.) and R. Russell (pers. comm.). ^{14}C-PCB 153 (Log K_{ow} = 6.9) was donated by Dr. K. Day (University of Windsor). The compound was originally purchased from Sigma Co. (St. Louis) at 12.6 Ci mol^{-1} in toluene but was

subsequently diluted in methanol to 5 μCi mL^{-1} and stored in 1 mL glass ampules. ^{14}C-labelled PCB 153 was prepared by swirling 225 μL in a slurry of 10 mL hexane and 3 glass wool boluses in a glass beaker (R. Russell, pers. comm.). The amount of PCB 153 used was based on its expected accumulation by the mussels and the detection limit of the radioactivity counter. The slurry was left to stand for 5 days to evaporate the hexane, leaving behind the PCB on the glass wool. Each bolus was wrapped in a larger bolus of uncontaminated glass wool and placed in a submersible fluvial filter. Each of these non-aerating filters was placed in a 36 L glass aquarium containing filtered lake water at 12°C. The system was allowed to stabilize for 5 - 7 days, at which time 45 zebra mussels were added. Following 4 days exposure, mussels were transferred to aquaria with clean water for 2 days to allow elimination of unassimilated PCB. Condition index was calculated for 5 mussels prior to and following the 6 day experiment. The three replicates were staggered 1 day apart.

Immediately following the clearing period, the remaining 40 mussels were placed individually in 20 mL scintillation vials containing 9 mL of filtered lake water and acclimated to 23°C for 1 hr. Half the mussels were induced to spawn (as detailed under Laboratory Protocol), while the others were treated similarly except 1 mL ropure water was added in place of the serotonin solution.

Radioactivity of gravid and spawned mussels was assessed using liquid scintillation counting. Mussels were carefully shucked, and shell and tissue placed into separate scintillation vials containing 5 mL of Ready-Solv scintillation cocktail (Beckman Instruments Inc., Fullerton, CA). Spawned eggs and sperm were gently filtered onto glass-fibre filters (0.7 μm GF/C; Whatman) and placed into 5 mL of scintillation cocktail. Samples, blanks, and background and ^{14}C quenched standards (0.097 mCi; Beckman) were counted (Beckman LS 3801) for 20 min at 80% confidence. Counts per minute (CPM) were converted to disintegrations per min (DPM) using the ^{14}C standard efficiency curve generated from counts of quenched standards. Preliminary tests showed

that Ready-Solv, a pseudocumene based cocktail, was able to effectively extract ^{14}C-PCB 153 from tissue without previous tissue digestion, and neither glass-fibre filters nor mass of tissue contributed to quenching.

This experiment was repeated with the following modifications: five times more PCB was added to a smaller amount of hexane (3 mL) and glass wool, the hexane/glass wool slurry was allowed to evaporate for 1 full wk, and once the fluvial filters were added to the aquaria, the system was allowed to stabilize for 2 wk. Also, an additional five mussels from each replicate were removed and counted immediately after chemical exposure (i.e. no clearing period) to determine if there uptake had in fact occurred.

DATA ANALYSES

Univariate and multivariate statistical analyses were conducted with reference to Sokal and Rohlf (1995), and Tabachnick and Fidell (1996), respectively. Analyses were performed using SYSTAT software (Wilkinson et al. 1992) and BMDP mainframe (Dixon 1992).

Reproductive Status and Egg Diameter

The frequency of female mussels at each of the 5 reproductive stages was recorded for each replicate. Because sex of mussels at stages 0 and 1 is not discernible, the numbers of mussels at these stages were adjusted to include females only by using the average sex ratio calculated from two dates when the sex of all mussels could be ascertained (see Results). The relationship between date and reproductive status was examined through a two-way chi-square test of association. Mean reproductive status for each replicate was calculated for multivariate analyses (see below). Mean egg diameter was also determined for each replicate using the mean egg diameter of each female. An Analysis of Variance (ANOVA) was used to test the effect of date on mean egg size.

Temporal Variation of Contaminants

Of the 49 chemicals quantified in zebra mussel tissue samples, 9 contaminants were selected to examine the effects of time, physiological status (i.e. zebra mussel lipid content and reproductive status), and limnological (i.e. temperature, chlorophyll a, and secchi depth) factors on chemical body burdens. Chemicals were chosen based on a complete temporal profile, relevance to published literature, and representative low- (HCB, PCB 31/28 and PCB 52), mid- (PCB 101, PCB 118 and PCB 138), and high- (PCB 153, PCB 180 and PCB 206) K_{ow} compounds. On two occasions, PCB 206 was not detected: 11 August - replicate 3 and 04 October - replicate 2. Random values between 0 and 0.1 $\mu g\ kg^{-1}$ (the detection limit for PCB 206) were substituted. Following $Log_{10}(value+1)$ transformation of all chemical concentrations, normal probability plots revealed 1 univariate outliers: HCB concentration for 25 May-replicate 3 was unusually high. Simply deleting this case would result in a loss of information regarding other variables during multiple regression (see below). As an alternative, the outlier value was replaced with the average of the original value and the next highest value. In this way the new value remained deviant, but not as deviant as the original value, thereby reducing the influence of the outlier (Tabachnick and Fidell 1996).

Missing values for Secchi depth (20 July and 4 October) and chlorophyll a (July 20) were estimated by maximum likelihood regression (Dixon 1992). Percent lipid content was arcsine(square root of proportion) transformed to improve normality. While mean reproductive status was positively skewed, inverse transformation would cause negative kurtosis; thus this variable was not transformed. All other independent variables were not transformed. Egg size was excluded from multivariate analyses due to inherent missing values (i.e. eggs are not present in immature and spent individuals) and non-significant variation in diameter for dates when eggs were present (see Results).

A multivariate regression model:

Chemical Concentrations = Julian Date + Spawning Cycle + Reproductive Status + % Lipid Content + Temperature + Chlorophyll a + Secchi Depth + (Reproductive Status X % Lipid) + (Reproductive Status X Temp.) + (% Lipid X Temp.)

was initially developed to examine temporal variation in contaminant concentrations. Because dependent and independent variables were highly correlated with one another, this procedure was wrought with difficulties. To simplify the model, two separate Principal Components Analyses (PCAs) were performed on dependent and independent variables. Through a linear combination of individual variables, PCA reduces the number of correlated variables to a few orthogonal factors (Tabachnick and Fidell 1996). The number of non-trivial factors was determined by the Kaiser-Guttman approach (i.e. factors with eigenvalues > 1). This criterion can over estimate the number of non-trivial components, but is considered suitable for matrices having strong correlation structure (Jackson 1993). PCA generates factor values for each case which represent individual factors scores had the factors been measured directly. To evaluate outliers, PCA provides the Mahalanobis distance of each case from the centroid of the factor scores evaluated as χ^2/d.f.; values larger than the critical χ^2 ($\alpha = 0.01$) are considered deviant. Factor values from both PCAs were used in multiple regression to examine the relationship between chemical concentrations (dependent factor) and seasonal, reproductive, lipid and transparency (independent factors) effects.

Effect of Spawning

The effects of spawning activity on lipid content and contaminant body burden were isolated by comparing percent lipid and chemical concentrations between gravid mussels and mussels spawned in the lab. Percent lipid, wet-mass concentrations, and lipid-adjusted contaminant concentrations were analyzed separately. Paired sample t-tests

were performed to test for a difference between male and female mussels. Alpha was adjusted from a value of 0.05 to 0.005 using the Dunn-Siduk method (Sokal and Rohlf 1995). Paired sample t-tests with the adjusted alpha value were also used to test the hypotheses that there were no significant differences in either percent lipid content or concentrations of the 9 selected contaminants (wet-mass and lipid adjusted) between gravid and spent mussels. Paired sample t-tests account for any confounding effect of date.

Lipid Groups

Percent total neutral lipid content was calculated by summing percents hydrocarbon, wax/sterol esters, methyl ester, triacylglycerol, free fatty acids, free aliphatic alcohol, free sterol, and diacylglycerol. Percent total polar lipid content was calculated by summing percents acetone-mobile polar lipids and phospholipids (see Appendix A). Temporal variation of % neutral lipids and % polar lipids was assessed with a factorial Multiple Analysis of Variance (MANOVA). The first factor was spawning cycle with 2 levels (first spawning cycle and second spawning cycle), while the second factor was reproductive status with 3 levels: immature, gravid, and spent mussels. Lipid contents were arcsine(square root proportion) transformed to normalize data prior to analysis.

Implications of different lipid adjustments to contaminant concentrations were assessed for individual chemicals. Log_{10}(value + 1) wet-mass chemical concentrations were regressed on the log_{10}(value + 1) of each lipid concentration for gravimetric total lipid (TLG) (as determined under Contaminant Analysis), Iatroscan total lipid (TLI), and Iatroscan neutral lipid (NLI). The three regressions were tested for significant differences in slope or intercept.

RESULTS

Reproductive Status and Egg Diameter

Zebra mussels spawned twice during the sampling period. The first spawning cycle began in mid - June and continued until early July; the second cycle occurred during the month of August (Figure 2). A χ^2 test of association between reproductive status and date was highly significant ($p<0.0001$). Sex ratio was determined on 13 June and 1 August; female mussels comprised 65.6 ± 1.1 and 61.1 ± 1.1% of the mussels collected on these dates, respectively. Eggs were observed in females from 25 May through 11 August. Mean egg diameter ranged from 54.8 ± 3.3 to 87.9 ± 3.5 μm but did not vary significantly by date (ANOVA: $F = 2.2$, d.f. = 7, 13, $p>0.05$).

Temporal Variation of Contaminants

Wet-mass concentrations of the 9 selected contaminants increased in June and declined slightly following the first spawning event in mid-July (Figure 3). Concentrations were maximal at the end July and declined steadily through the remainder of the study period. Lipid-adjusted concentrations varied little within a spawning cycle, but concentrations were higher during the second spawning cycle than the first (Figure 4). Concentrations were highest for moderately high-K_{ow} PCBs with 40 - 60% chlorination. For example, PCB 138 and PCB 153 had the highest concentrations, while PCB 206 and HCB had the lowest (Figures 3 and 4).

Water temperature increased from 11°C on 12 May to a maximum of 30°C on 11 August, from which it declined to 15°C by 4 October. Chlorophyll a content peaked twice: first on 20 June at 8.4 mg m^{-3} and again on 11 August at 8.2 mg m^{-3}. No chlorophyll a was detected on 12 May or after 21 August. Secchi depth ranged between 1.5 and 4.0 m and had a mean of 2.68 ± 0.13 m; no seasonal pattern was evident.

Principal component analysis of chemical concentrations (dependent variables) produced 1 factor (PC) on which variable loadings were proportional to mean chemical concentrations (Table 2). This factor had an eigenvalue of 7.23 and accounted for 80% of the total variance. HCB had the smallest factor loading (0.645) while PCB 153 had the largest (0.987). For one factor, the Mahalanobis distance critical χ^2 is 6.63 ($\alpha = 0.01$); no cases produced scores that made them outliers in the space of the solution. Variables were generally well-defined by the factor solution; communalities of the variables (i.e. variance accounted for by the factor) tended to be high and factor loadings were large (Table 2). Because there was only one factor, no rotation of the solution was necessary. Hereafter, the PC will be referred to as chemical concentration factor (CC).

PCA reduced the independent variables to 3 principal components (PCs) (Table 3). Date and spawning cycle contributed positively while lipid contributed negatively to PC1. PC2 was comprised of reproductive status variables, and PC3 of temperature, chlorophyll a, and Secchi depth (negative contribution). The 3 factors accounted for 89.5% of the total variance, with factors 1 and 2 contributing ~32% each. All variables loaded on to a single factor, except for temperature which loaded onto two factors (PC1 and PC3). Hereafter, the three factors will be referred to as seasonal (S), reproduction (R), and limnological (L), respectively. Varimax rotation produced the best separation of these factors. No case produced a Mahalanobis distance score greater than the critical χ^2 for three factors of 3.78 ($\alpha = 0.01$). The three factors were well-defined by the variables as indicated by squared multiple correlations equal to one for each factor with the variables. Variables were also well-defined by the factor solution suggested by high communality values. Independent variables were considered meaningful contributors to PCs when loadings were >0.55 (30% overlapping variance).

Standard multiple regression analysis was performed between CC as the dependent variable and S, R, and L as the independent variables. The multiple correlation coefficient for the regression was significantly different from zero ($F = 14.40$, d.f. = 3, 29,

$p<0.05$). All factors contributed equally to the prediction of chemical concentration (Table 4).

Effect of Spawning

No significant differences in percent lipid content or chemical concentrations between spent male and female mussels were detected by paired comparisons (Paired-sample t-tests: adjusted $\alpha = 0.005$). As such, spent male and female concentrations were averaged for each replicate. No significant differences in percent lipid content or chemical concentrations (wet-mass and lipid-adjusted) between gravid and spent mussels were found (paired-sample t-tests: adjusted $\alpha = 0.005$) (Table 5, Figure 5).

Lipid Groups

Iatroscan analysis showed high levels of free fatty acids which are indicative of hydrolysis of lipid components (e.g. triacylglycerol) possibly due to reaction with methanol during extraction and/or storage of lipid extracts (Appendix A, Fogerty 1971; Gardner et al. 1985). Thus, while examination of individual lipid classes was inappropriate, lipids were pooled into polar and neutral groups (Table 6). Both spawning cycle and reproductive status significantly affected total neutral lipid content ($F = 25.26$ and 4.79, respectively, d.f. = 1, 12, $p<0.05$; MANOVA) but not total polar lipid content ($F = 3.32$ and 2.05, respectively, d.f. 1, 12, $p>0.05$). The effect of reproductive status was dependent on spawning cycle ($F = 18.52$, d.f. = 2, 12, $p<0.05$).

Concentrations of all but two chemicals (HCB and PCB 31/28) were significantly correlated to neutral lipid content (NLI) (Table 7). Coefficient of determination was highest for PCB 118 ($R^2=0.66$) and lowest for PCB 180 ($R^2=0.37$). Total lipid quantified gravimetrically (TLG) or by Iatroscan (TLI) correlated poorly with all chemicals except PCB 118. Contaminant concentrations normalized to neutral lipid content gradually

increased from May through August for moderate K_{ow} compounds (Figure 6) in contrast to concentrations based on wet-mass (Figure 3) and total lipid-mass (Figure 4).

Partitioning of Radio-labelled PCB

Condition indices of experimental zebra mussels did not decline significantly during ($F = 1.40$ and 4.61, d.f. = 2, 12, $p>0.05$; ANOVAs) or between ($F = 0.10$, d.f. = 1, 28, $p>0.05$; ANOVA) studies. The overall mean condition index was 0.05 ± 0.002. Scintillation counting of shells and tissues of exposed mussels (from both attempts) reported DPMs well within background range (20-50 DPM). Thus, both radio-tracer studies failed to reveal significant uptake of contaminant by mussels in the laboratory.

DISCUSSION

The reproductive cycle of the zebra mussel population surveyed in this study was not unusual. For example, two spawning events within a season that were observed here have been reported for other European and North American populations (Borcherding 1991; Mackie 1993). That egg size did not vary significantly with time was surprising but not unique (D. Garton, pers. comm.). The population was skewed more toward females (61.1-65.6 %) than is typically observed, however. For example, Garton and Haag (1993) previously reported populations in western Lake Erie with equal in sex ratios, though Walz (1978) found European populations slightly biases toward females (55.9%).

Total lipid levels reported here are well within published ranges (1.2 - 1.8% wet-weight) (Brieger and Hunter 1993; van der Oost et al. 1988). As well, the temporal variation in total lipid content in zebra mussels shown here is consistent with observations made in Lake St. Clair in 1990 and 1991 (Nalepa et al. 1993). Researchers generally attribute changes in lipid content to reproductive activity though no direct measurement of the latter has been made (Pieters et al. 1980; Zandee et al. 1980; Nalepa et al. 1993.). In this study, the relationship between lipid content and reproductive status was less direct. Mussels spawned synchronously twice during 1995, yet total lipid and neutral lipid content, as determined by Iatroscan, were maximal immediately following the first spawning event (i.e. when mussels were spent; Table 6). Lipid levels declined steadily thereafter despite a second cycle of gametogenesis and spawning (Figure 2). Moreover, induced spawning in the lab revealed no significant difference in lipid content between spent male and female mussels or between gravid and spent mussels. This finding is perplexing considering that zebra mussels can lose up to 50% of their dry mass during the release of gametes (Sprung 1991; Garton and Haag 1993). Borcherding (1991) monitored zebra mussels from Fühlinger See and Heider Bergsee, Germany, and found that the gonad index (gonad volume/total soft tissue volume) peaked at 50% and declined

to less than 30% following spawning. The absence of such a relationship in this study may have resulted from either insufficient gametes shed to detect a difference or that the gametes contained an insignificant amount of lipid compared to the whole animal. Sprung (1989) found the lipid content of zebra mussel eggs to be highly variable ranging from 9.8 to 78.8 % of dry-mass. Lipid levels, for mussels with low-lipid eggs, would be most strongly governed by other physiological or environmental factors rather than by spawning activity.

Regardless of how lipid content and reproductive activity interact, both were positively correlated to PCB concentration. That lipid content and reproductive status separated on different Principal Components (Table 3) suggests they influence contaminant concentrations differently but, based on standardised regression coefficients (Table 4), are of equal importance. Again, the effect of reproductive status did not appear to be directly related to spawning activity. It should be noted that because PCA is not generally recommended for data sets where the sample size is less than fifty, results must be interpreted with caution. It is my belief, however, that because the results conformed with a "good" PCA and the PCA simplified additional analyses, this procedure was justified.

Contrary to this study, previous work has demonstrated that fluctuations in dry weight concentrations of PCBs 28/31 and 95 in the blue mussel Mytilus edulis corresponded with gametogenesis and spawning (Capuzzo et al. 1989). Further work with Mytilus revealed that PCB concentration in the mantle, which includes reproductive tissues, accounted for up to 40% of total PCB body burden (Hummel et al. 1989). The authors speculated that a substantial proportion of contaminants may be shed during spawning of gametes. Changes in organochlorine contaminants due to gametogenesis and spawning has also been reported in fishes. For example, Guiney et al. (1979) observed a redistribution of tetrachlorobiphenyl during egg formation and enhanced elimination when gametes were spawned from rainbow trout Oncorhynchus mykiss

(formally Salmo gairdneri). Furthermore, approximately 40% of total PCB body burden was eliminated through spawning in female Chinook salmon Oncorhynchus tshawytscha from Lake Michigan (Miller 1994). Westin et al. (1983) also reported that spawning in female striped bass Morone saxatilis eliminated PCBs from body tissues. That no changes in wet-weight PCB concentrations occurred as a result of spawning in zebra mussels may indicate that significant amounts of PCBs did not accumulate in the gametes, perhaps due to the a low lipid content, or that a insufficient mass of gametes was shed during spawning to detect a difference. Moreover, under field conditions, the anticipated decrease in wet-mass contaminant concentrations following a spawning event was observed only for the first spawning cycle. It is thus possible that other environmental influences may obscure the effect of spawning. As determined here, temperature, chlorophyll a and water clarity significantly influenced contaminant concentration.

Changes in lipid content can potentially have profound effects on contaminant uptake and elimination kinetics. Laboratory studies examining the kinetics of organochlorine compounds generally employ non-reproductive mussels, of which lipid levels remain constant in a controlled environment. Under these conditions, kinetics are considered to be rapid (Morrison et al. 1996; Fisher et al. 1993). For example, time to essential steady state for organochlorine contaminants in zebra mussels ranges from 20 to 85 days with half-lives ranging from 9 to 26 days (Pruell et al. 1986; Tanabe et al. 1987b; Kannan et al. 1989; Nelson et al. 1995; Morrison et al. 1996). Bruner et al. (1994) determined that, in general, uptake rates were greater and elimination rates lower for high-lipid than for low-lipid Dreissena.

However, zebra mussels can substantially alter their biochemical make-up in the time required to achieve equilibrium conditions. I calculated that in less than 2 months, mussels could deplete their total lipid stores by approximately 40% (i.e. % difference between 03 July and 21 August; Table 6); this value increases to 70% if only neutral

lipids are considered. When the capacity to hold contaminants decreases faster than the contaminants can be eliminated, the result is a non-equilibrium condition in which the fugacity of the organism is greater than that of its environment. This condition could cause over-estimation of bioconcentration factors and perhaps altered contaminant profiles of some chemicals (e.g. high K_{ow} compounds) that are differentially retained due to slow elimination rates. Chevreuil et al. (1995) reported that lipid-adjusted PCB levels in roach (Rutilus rutilus)were lower in autumn, when lipid content was highest, than in spring. Similarly, Hummel et al. (1990) noted that on a fat (neutral lipid) basis, PCB concentration decreased with increasing fat content. Although Hummel et al. (1990) concluded that fat content did not determine PCB concentration, it may well be that the lipid content in both these species increased more rapidly than uptake kinetics could accommodate. Hence, annual fluctuations in lipid levels and reproductive state may limit mussels to achieving only quasi-steady-state conditions (Pizza and O'Connor 1983; Capuzzo et al. 1989). Given a possible 70% decrease in contaminant capacity, the potential shift in steady-state conditions is likely to be significant. Further, as lipid levels are known to affect uptake and elimination kinetics (Bruner et al. 1994), individuals monitoring *in situ* bioconcentration of contaminants must be cognizant of any lipid changes, and subsequently account for these changes in estimated time to equilibrium. Mussels deployed in the spring when lipid levels are increasing will require more time to establish equilibrium conditions than mussels deployed in the late summer when lipid levels are decreasing. Moreover, because the influence of reproductive status is still poorly understood, *in situ* biomonitoring programs should take place prior to any lipid increases in May or after the reproductive period. At a minimum, lipid levels and reproductive state of mussel 'biomonitors' should be monitored.

The debate on lipid normalization of contaminant concentrations has entered its third decade (Holden 1962). Most workers now agree that for non-ionic, hydrophobic compounds such as PCBs, the primary depot is the lipid fraction of the organism. Thus

the contaminant concentration must be corrected for the lipid content of the organism or phase. The ratio technique, in which the contaminant concentration is simply divided by the lipid concentration, is most often applied. Hebert and Keenleyside (1995) compared this approach to Analysis of Covariance (ANACOVA) and found the two techniques yielded significantly different results. However, their examples were based on small samples of hypothetical data, and perhaps more importantly, they failed to consider the possibility of compositional differences in lipid content between experimental units. There is growing evidence that lipid pools, either between species or between tissues within an individual, cannot be treated as a static, homogeneous phase. If the lipid fraction is homogeneous in nature, and the organism is in equilibrium with its environment, lipid-adjusted contaminant concentrations (fugacities) should be equal regardless of species, tissues or reproductive condition of the organism. Yet this pattern seems to be more the exception than the rule. de Kock (1983) detected higher lipid-adjusted PCB concentrations in M. edulis in winter than during summer. van der Oost et al. (1988) determined that, on a wet-weight basis, PCB concentration increased with trophic level, but found no consistent pattern on a lipid normalized basis. Capuzzo et al. (1989) recorded a substantial decline in wet-weight PCB concentrations while lipid-adjusted levels generally increased in Mytilus following spawning activity; a change in overall contaminant profile was also noted after spawning. This suggests that smaller amounts of specific PCB congeners were eliminated than had been expected based on equilibrium partitioning. The authors suggested differential partitioning of specific PCB congeners into different tissues or lipid pools. Chevreuil et al. (1995) also observed that contaminant profiles varied greatly between the roach, which accumulated mainly penta-CBs, and zebra mussel, which accumulated mainly tri- and tetra-CBs. In this study, zebra mussels accumulated penta-and hexa-CBs to a greater degree than tri- and tetra-CBs. Pelliner et al. (1994) and Hühnerfuss et al. (1995) speculated that when seasonal changes in contaminant body burden are not removed from lipid normalization, observed patterns

of variation are actually due to changes in lipid composition rather than total lipid content.

Indeed, lipid composition is known to vary between species and, seasonally, within species (Delbeke et al. 1995). Hummel et al. (1990) determined that the ratio of neutral lipids to total lipids in Mytilus was in a constant state of flux, that tissues varied widely in lipid composition, and that the mantle and digestive gland were relatively rich in neutral lipids compared to gills (Lubert et al. 1985). Lean fish such as perch (Perca fluviatilis) and cod (Godus morhua) tend to have a large proportion of phospholipids, while fatty fish such as Atlantic salmon (Salmo salar) are rich in storage lipids (Ewald and Larsson 1994). Polar lipids are dominant in zebra mussels, as neutral lipids comprised only 20-30% of total lipids and <1% of total wet weight (Table 6). Naiepa et al. (1993) similarly found phospholipids to be dominant in zebra mussels from Lake St. Clair, while triacylglycerols were the most important neutral lipid.

Neutral and polar lipid components vary in solubility, other molecular characteristics, and in contaminant kinetics (Ewald and Larsson 1994). For example, the lipid-water partitioning of low K_{ow} TCB (PCB 47) was significantly greater for neutral triacylglycerols than for polar phospholipids (Ewald and Larsson 1994). Concentrations of chlorobenzenes (log K_{ow} = 3.5 - 5.2) were also 2 orders of magnitude higher in neutral lipids than polar lipids (van Weel and Opperhuizen 1995). Thus, non-ionic hydrophobic contaminants preferentially partition into tissues that are relatively rich in storage lipids as opposed to structural lipids. It seems only logical to examine the relationship between non-ionic hydrophobic contaminants and neutral lipid content more closely. Delbeke et al. (1995) observed differences in functional relationships between PCBs and lipids when different lipid quantification methods were compared. Normalizing PCB concentrations on neutral lipid content, as quantified by Iatroscan, explained 50% of the variability between species irrespective of their trophic status. Similar correlations (R^2=0.37 to 0.66) between PCB (log K_{ow} >5.71) concentration and neutral lipid content of zebra

mussels were attained in this study. Equilibrium partitioning may, therefore, depend more on neutral than on total lipids. Although hydrophobicity is the driving force behind partitioning dynamics, contaminant distribution and concentration ultimately attained within an organism is governed by neutral content. Consequently, changes in neutral lipid content may explain anomalies in lipid-adjusted contaminant concentrations. For example, Nelson et al. (1995) found a decrease in contaminant level, based on dry weight, of deployed Mytilus, but without a corresponding change in lipid content. It is possible that the proportion of neutral lipid decreased with an equivalent increase in polar lipid, such that total lipid remained unchanged. Further evidence was provided by Hummel et al. (1989), who detected no difference in PCB concentrations on a fat (neutral lipid) basis between various tissues of individual blue mussels (Mytilus).

Normalization of hydrophobic contaminants to neutral lipid content may also explain why the relationship between BCF and K_{ow} departs from linearity for high K_{ow} compounds. Proposed reasons for the non-linear nature of this relationship include steric hindrance, non-equilibrium conditions, and decreased lipid solubility (Connell 1988). Additionally, BCFs based on neutral lipid content rather than total lipid content may more accurately reflect potential bioconcentration abilities. Furthermore, the assumption that octanol is an appropriate surrogate for lipid needs to be revisited because Chiou (1985) has demonstrated that triolein is likely a better model for lipid than is octanol.

Compositional changes in lipid content, however, cannot explain the increase in lipid-adjusted contaminant body burdens seen during the second spawning cycle in this study. Even when contaminant concentrations were adjusted on the basis of neutral lipid content, they remain elevated (Figure 6). The increased burden may be due to increased PCB bioavailability between 03 July and 20 July caused by re-suspension of sediments during severe storm activity between 13 July and 18 July. According to the Great Lakes Forecasting System integrated water currents increased from 0.16 km hr^{-1} to 0.25-0.37 km hr^{-1} during this period (GLFS 1996). Low K_{ow} compounds are best able to monitor

contaminant pulse events in the environment because they are able to achieve equilibrium conditions rapidly. In this study, neither HCB nor PCB 31/28 show elevated lipid-adjusted contaminant on 20 July. If a pulse of contaminants did occur between 03 July and 20 July, they may have depurated from the mussels given that time to steady state for these contaminants is approximately 20 days. Concentrations of PCB 52 were however elevated on 20 July despite similar lipid composition and content to earlier sampling dates. Time to steady state for this compound is approximately 28 days. Thus, this compound may most accurately reflect the contaminant profile of the environment. That concentrations remained high through to 21 August may be a consequence of both total and neutral lipid contents declining faster than contaminants could be eliminated. Seasonal variation in lipid concentration and/or composition can therefore confound actual changes in contaminant concentrations occurring in the environment.

SUMMARY

Zebra mussels in the western basin of Lake Erie spawned twice during the summer of 1995, first in June and again in August. The relationship between reproductive status and lipid content was unclear Total lipid content was maximal following the first spawning event and declined steadily thereafter despite a second cycle of gametogenesis and spawning. Results from Principal Components Analysis and Multiple Regression suggested that lipid content and reproductive status affect contaminant body burdens, though in different ways. That induced spawning of zebra mussels affected neither total lipid content nor contaminant levels suggests that gonads do not store a large proportion of the lipid or contaminant load. Therefore, while reproductive status clearly affects contaminant dynamics, the effect is not related simply to changes in total lipid content or to spawning itself.

Lipid composition can also be important in determining concentrations of hydrophobic contaminants. PCBs ($K_{ow} > 5.71$) were more highly correlated with neutral lipid content than total lipid content. Consequently, temporal changes in neutral lipid content within an individual or lipid composition differences between species may explain why some lipid-adjusted contaminant concentrations and BCFs depart from those predicted by equilibrium partitioning.

Figure 1: Location of Dreissena sampling site in western Lake Erie. Latitude and longitude are N41°57.260 and W83°14.114, respectively. Lengths of the three transects are not to scale.

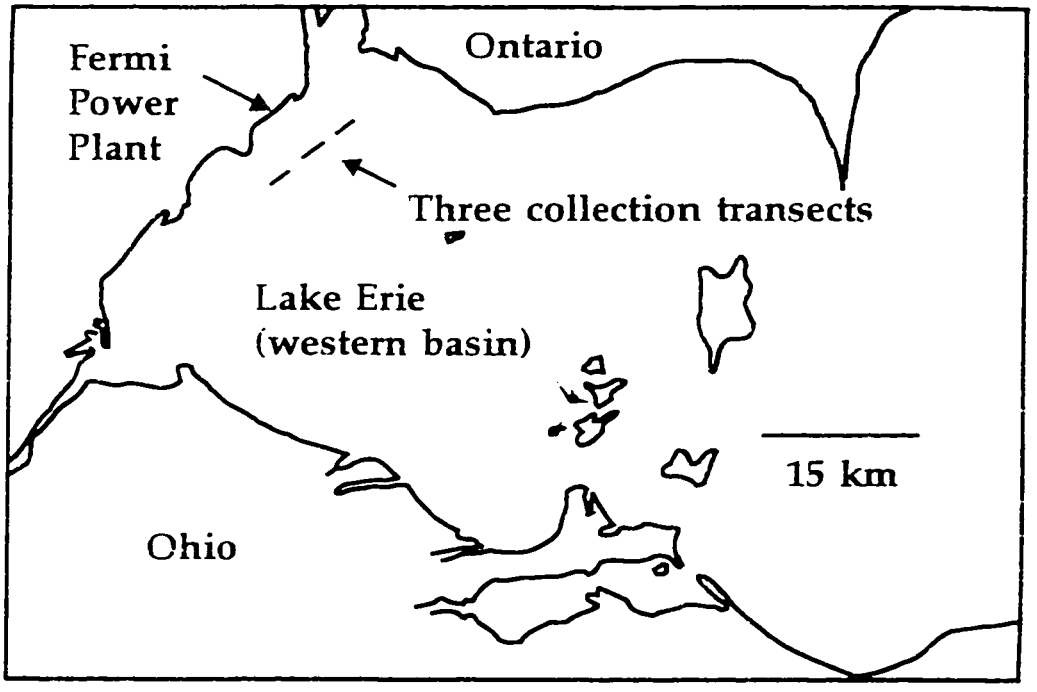
Fermi
Power
Plant
Ontario
Three collection transects
Lake Erie
(western basin)
15 km
Ohio

Figure 2: Mean (± S.E.) % lipid content (solid circles) of zebra mussels and mean (+ S.E.) reproductive status (open bars) of female zebra mussels where: 1 = immature, sexes indistinguishable; 2 = immature, sexes separate; 3 = germinal vesicle in < 50% of eggs; 4 = germinal vesicle in > 50% of eggs; 0 = spent.

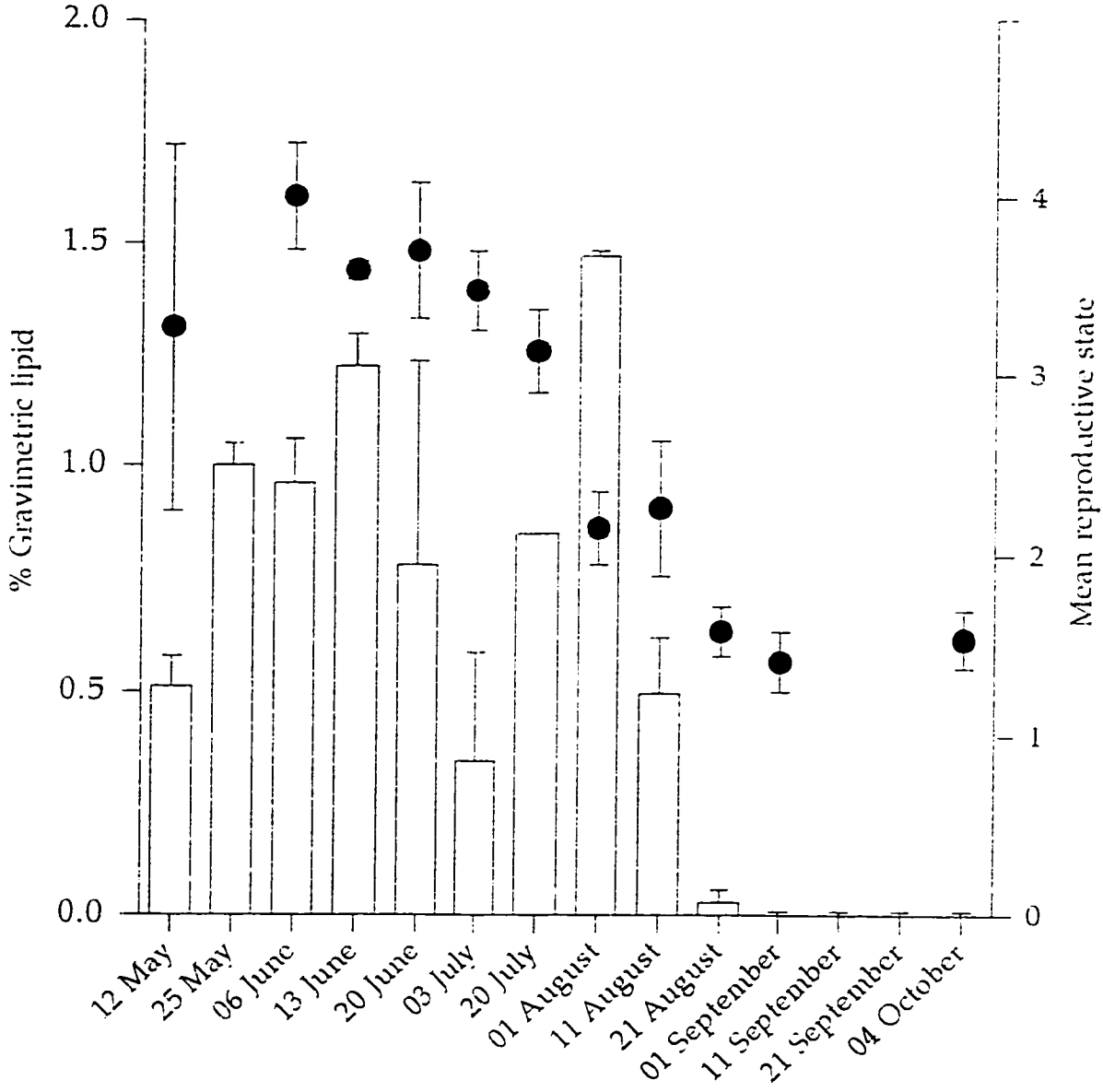
2.0
1.5
1.0
0.5
0.0
% Gravimetric lipid
4
3
2
1
0
Mean reproductive state
12 May
25 May
06 June
13 June
20 June
03 July
20 July
01 August
11 August
21 August
01 September
11 September
21 September
04 October

Figure 3: Temporal variation in mean (+ S.E.) wet-mass contaminant concentrations of each of 3 low-, mid-, and high-K_{ow} compounds. The six dates represent sexually immature, gravid and spent mussels for the two spawning cycles. Note the concentration scales are dissimilar.

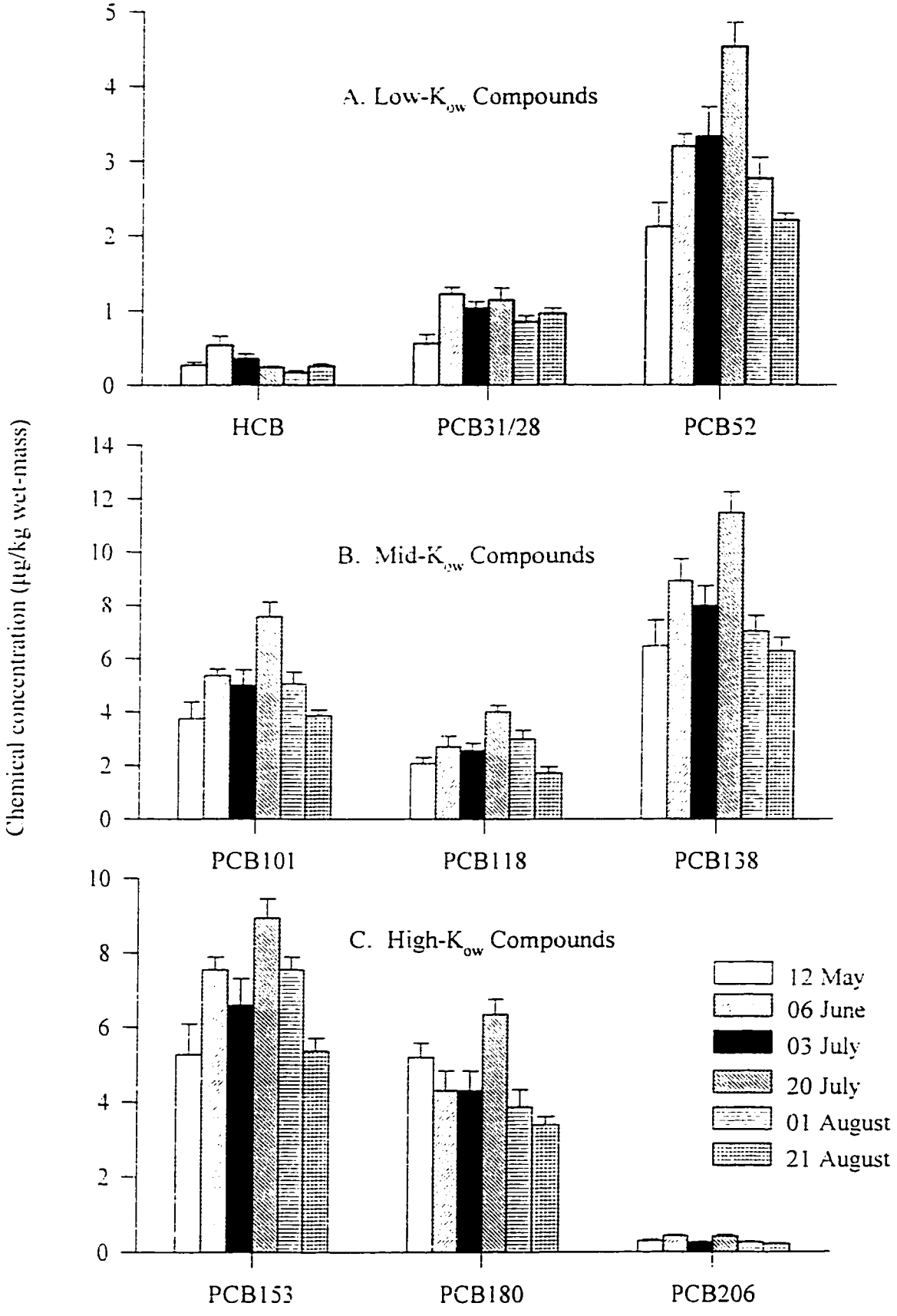
A. Low-K_{ow} Compounds
B. Mid-K_{ow} Compounds
C. High-K_{ow} Compounds
Chemical concentration (µg/kg wet-mass)
HCB
PCB31/28
PCB52
PCB101
PCB118
PCB138
PCB153
PCB180
PCB206
12 May
06 June
03 July
20 July
01 August
21 August

Figure 4: Temporal variation in mean (± S.E.) lipid-adjusted contaminant concentrations of each of 3 low-, mid-, and high-K_{ow} compounds. The six dates represent sexually immature, gravid and spent mussels for the two spawning cycles. Note the concentration scales are dissimilar.

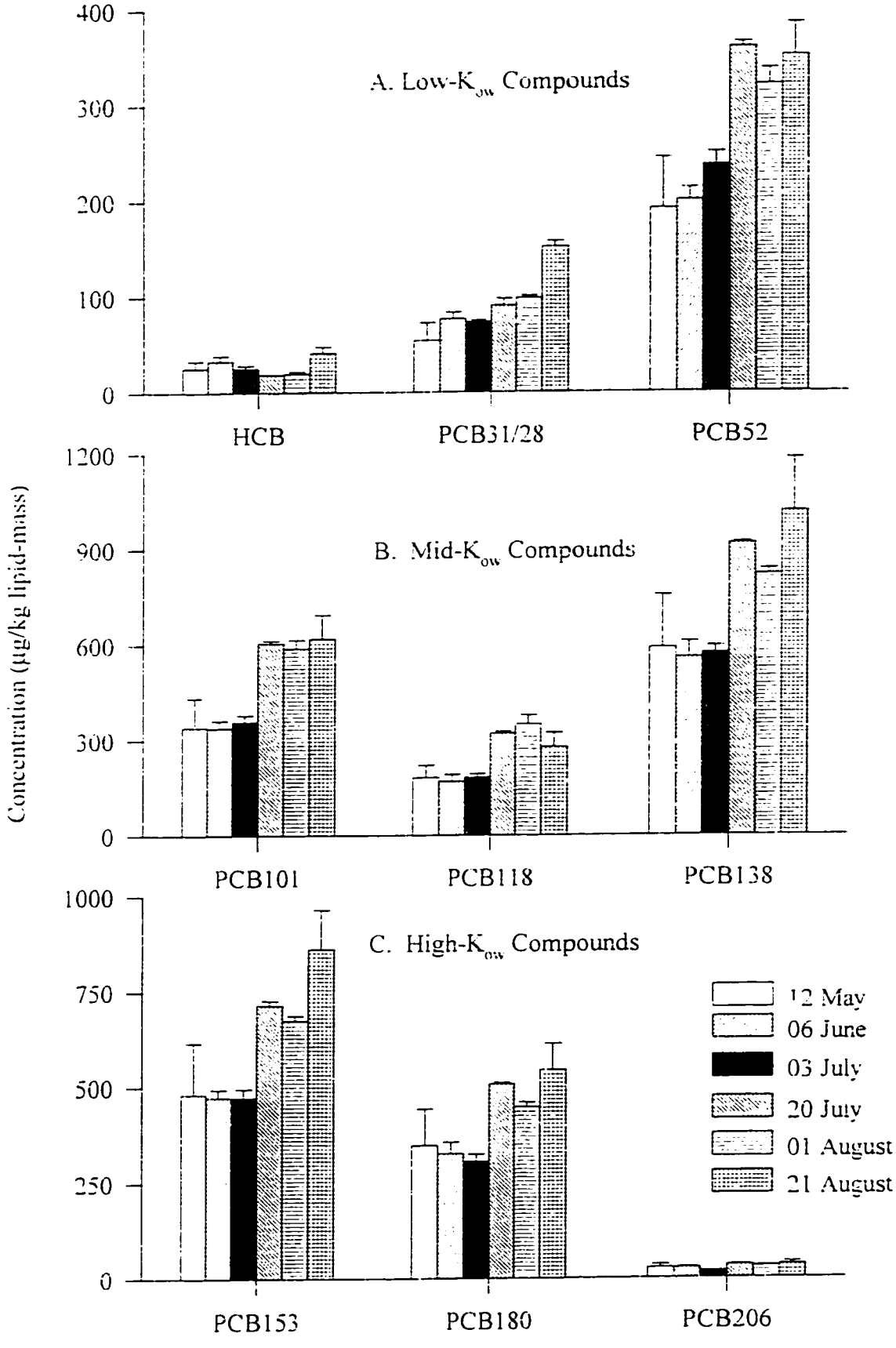
A. Low-K_{ow} Compounds
B. Mid-K_{ow} Compounds
C. High-K_{ow} Compounds
Concentration (µg/kg lipid-mass)
400
300
200
100
0
1200
900
600
300
0
1000
750
500
250
0
HCB
PCB31/28
PCB52
PCB101
PCB118
PCB138
PCB153
PCB180
PCB206
12 May
06 June
03 July
20 July
01 August
21 August

Figure 5: Differences in mean (± S.E.) contaminant body burdens between gravid (open bars) and spent (shaded bars) mussels. Panels A and B show concentrations based on wet-mass and lipid-mass, respectively. Differences in % lipid are shown in the insert in Panel A.

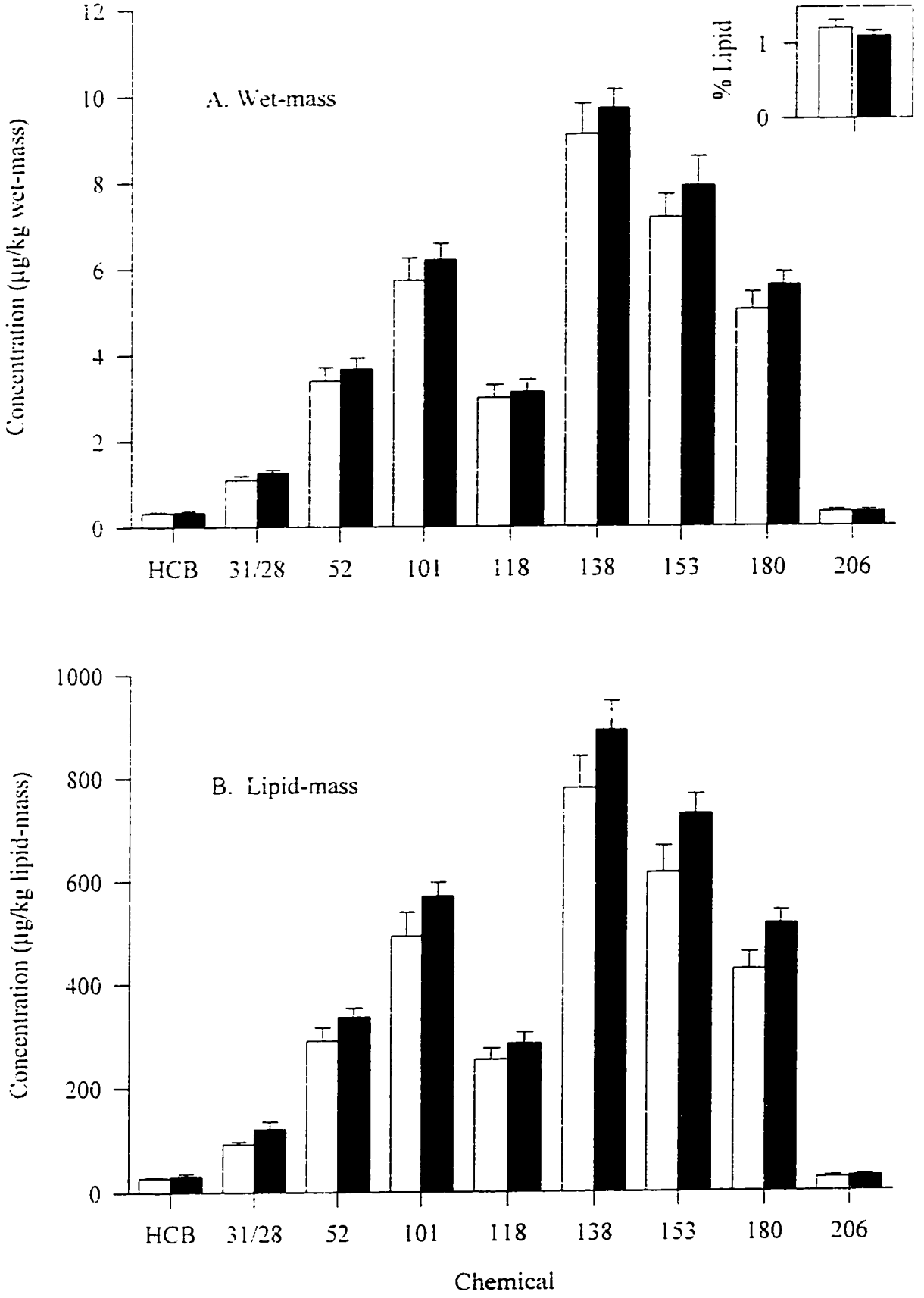
A. Wet-mass
% Lipid
Concentration (µg/kg wet-mass)
0
2
4
6
8
10
12
0
1
HCB
31/28
52
101
118
138
153
180
206
B. Lipid-mass
Concentration (µg/kg lipid-mass)
0
200
400
600
800
1000
HCB
31/28
52
101
118
138
153
180
206
Chemical

Figure 6: Temporal variation in mean (± S.E.) contaminant concentrations normalized to neutral lipid content of each of 3 low-, mid-, and high-K_{ow} compounds. The six dates represent sexually immature, gravid and, spent mussels for the two spawning cycles. Note the concentration scales are dissimilar.

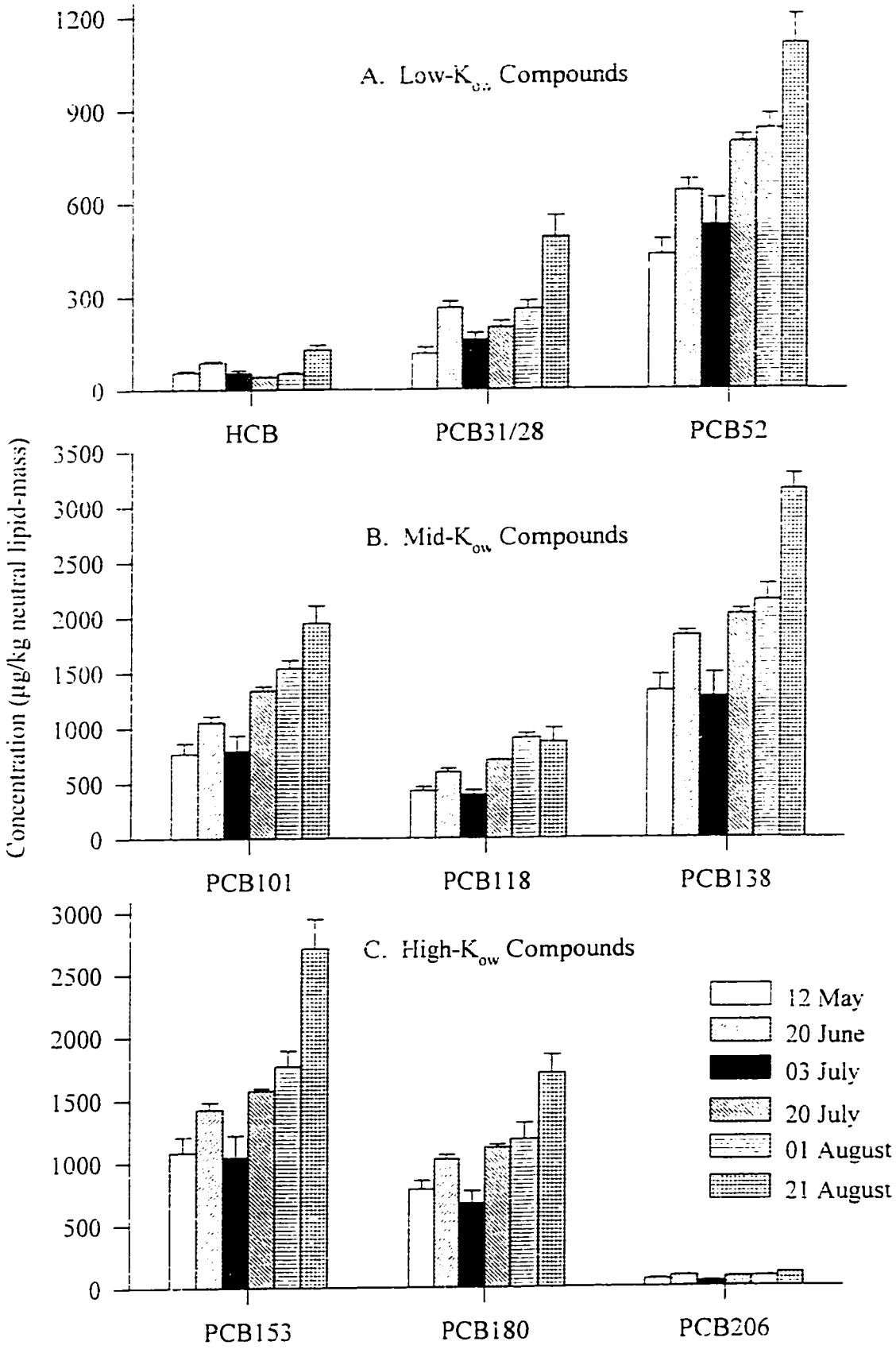
A. Low-K_{ow} Compounds
B. Mid-K_{ow} Compounds
C. High-K_{ow} Compounds
Concentration (µg/kg neutral lipid-mass)
1200
900
600
300
0
3500
3000
2500
2000
1500
1000
500
HCB
PCB31/28
PCB52
PCB101
PCB118
PCB138
PCB153
PCB180
PCB206
12 May
20 June
03 July
20 July
01 August
21 August

Table 1: List of sample collection and analyses (Yes/No) dates.

Collection Date (1995)	Julian Date	Contaminant Analysis?	Lipid Analysis by Iatroscan?
12 May	132	Y	Y
25 May	145	N	N
06 June	157	Y	N
13 June	164	Y	N
20 June	171	Y	Y
03 July	184	Y	Y
20 July	201	Y	Y
01 August	213	Y	Y
11 August	223	Y	N
21 August	233	Y	Y
01 September	244	Y	N
11 September	254	N	N
21 September	264	N	N
04 October	277	Y	N

Table 2: Hexachlorobenzene (HCB) and PCB congeners (identified by IUPAC No.) selected for statistical analysis; log K_{ow}, number of chlorine atoms, mean concentration pooled across replicates and dates (mg kg^{-1} wet-mass) ± S.E., rank of mean concentration, and factor loadings and communalities (i.e. variance of variables accounted for by the factor; h^2) from Principal Components Analysis. Factor loadings less than 0.55 (30% overlapping variance) were reduced to zero.

Chemical	Log K_{ow}	No. of Cl Atoms	Mean Conc. ± S.E.	Mean Conc. Rank	Factor Loadings	h^2
PCB 153	6.92	6	6.09 ± 0.23	2	0.987	0.9733
PCB 138	6.83	6	7.45 ± 0.34	1	0.978	0.9569
PCB 180	7.36	7	4.17 ± 0.19	4	0.978	0.9556
PCB 52	5.84	4	2.71 ± 0.15	5	0.968	0.9374
PCB 101	6.39	5	4.62 ± 0.23	3	0.962	0.9263
PCB 206	7.84	9	0.27 ± 0.02	9	0.856	0.7331
PCB 118	6.74	5	2.35 ± 0.14	6	0.840	0.7054
PCB 31/28	5.71	3	0.94 ± 0.05	7	0.788	0.6213
HCB	5.5	6	0.30 ± 0.02	8	0.645	0.4159

Table 3: Communalities (i.e. variance of the variables explained by the factors: h^2) and factor loadings from Principal Components Analysis of independent variables. Factor loadings less than 0.55 (30% overlapping variance) were reduced to zero. The percent of the total variance explained by each factor is shown in the last row.

Independent Variable	h_i^2	Factor 1 "Seasonal"	Factor 2 "Reproduction"	Factor 3 "Limnological"
Cycle	0.921	0.948	0.000	0.000
Date	0.8697	0.912	0.000	0.000
Lipid	0.9832	-0.804	0.000	0.000
Reproduction	0.9954	0.000	0.952	0.000
Reproduction X Temperature	0.9801	0.000	0.936	0.000
Reproduction X Lipid	0.9644	0.000	0.920	0.000
Secchi Depth	0.8718	0.000	0.000	-0.848
Chlorophyll a	0.9233	0.000	0.000	0.791
Lipid X Temperature	0.9421	0.000	0.000	0.790
Temperature		0.671	0.000	0.676
Variance Explained		32.0%	31.4%	26.2%

Table 4. Regression coefficients (B), standardized correlation coefficients (*B*), and intercept from standard multiple regression of seasonal, reproduction, and limnological factors on chemical concentration.

Variables	B	*B*
Seasonal	-0.4123	-0.41***
Reproduction	0.4697	0.47***
Limnological	0.4545	0.45***
Intercept	-0.00002	
R =	0.773	
R^2 =	0.597***	

***p<0.001.

Table 5: Paired-sample t-tests (adjusted $\alpha = 0.005$) between gravid and spent mussels for % lipid content, wet-mass and lipid-adjusted contaminant concentrations

Chemical	Wet -mass t-value	Wet -mass p-value	Lipid- adjusted t-value	Lipid- adjusted p-value
PCB 153	-1.433	0.190	-2.778	0.024
PCB 138	-0.895	0.397	-2.169	0.062
PCB 180	-1.626	0.143	-2.737	0.026
PCB 52	-1.195	0.266	-2.754	0.025
PCB 101	-1.153	0.282	-2.464	0.039
PCB 206	-0.066	0.949	-1.126	0.293
PCB 118	-0.451	0.664	-1.098	0.304
PCB 31/28	-1.571	0.155	-3.052	0.016
HCB	-0.730	0.486	-1.935	0.089
% Lipid	1.074	0.314	----	----

Table 6: Mean (± S.E.) percent of total lipid (whole animal wet-mass basis) of neutral (NLI), polar (PLI) and total lipids (TLI) as quantified by Iatroscan analysis. The six dates represent sexually immature, gravid, and spent mussels from each spawning cycle.

Date	Reproductive Category	% NLI	% PLI	% TLI
12 May	immature	0.48 ± 0.02	0.75 ± 0.02	1.23 ± 0.02
20 June	gravid	0.48 ± 0.03	0.87 ± 0.03	1.35 ± 0.06
03 July	spent	0.67 ± 0.12	0.81 ± 0.05	1.48 ± 0.17
20 July	immature	0.57 ± 0.03	0.82 ± 0.03	1.39 ± 0.08
01 August	gravid	0.32 ± 0.02	0.77 ± 0.06	1.10 ± 0.07
21 August	spent	0.20 ± 0.01	0.67 ± 0.03	0.87 ± 0.04

Table 7: Standard linear regression of contaminant concentration (wet-mass) on each lipid measurement: gravimetric total lipid (TLG), Iatroscan total lipid (TLI), and Iatroscan neutral lipid (NLI). F-ratio tests for differences between lipid groups.

Chemical		TLG	TLI	NLI	F Ratio
PCB 153	B	2.02	4.35	4.98**	2.92*
	Intercept	0.71	0.45	0.59	
	R^2	0.15	0.18	0.44**	
PCB 138	B	1.89	3.88	4.43**	2.64*
	Intercept	0.64	0.42	0.55	
	R^2	0.15	0.12	0.40**	
PCB 180	B	1.78	3.17	4.18**	2.21
	Intercept	0.51	0.36	0.43	
	R^2	0.15	0.11	0.37**	
PCB 52	B	2.56	5.30*	5.34**	3.62*
	Intercept	0.31	-0.01	0.22	
	R^2	0.21	0.24	0.46**	
PCB 101	B	2.45	5.56*	5.85**	3.71**
	Intercept	0.49	0.14	0.37	
	R^2	0.18	0.24*	0.49**	
PCB 206	B	0.84	1.16	1.74**	2.54
	Intercept	0.01	-0.03	-0.02	
	R^2	0.20	0.10	0.41**	
PCB 118	B	3.19**	6.36**	6.28**	6.97***
	Intercept	0.20	-0.16	0.12	
	R^2	0.36**	0.35**	0.66***	
PCB 31/28	B	-0.10	0.18	0.65	0.08
	Intercept	0.29	0.26	0.23	
	R^2	0.00	0.07	0.02	
HCB	B	0.2324	0.44	0.23	0.17
	Intercept	0.07	0.05	0.08	
	R^2	0.03	0.02	0.01	

*p<0.05; **p<0.01; ***p<0.001

APPENDIX

Lipid class composition as mean (± S.E.)% of total lipid quantified by Iatroscan analysis

Lipid Class	12 May	20 June	03 July	20 July	01 August	21 August
hydrocarbon	1.52±0.16	1.24±0.03	0.49±0.49	1.86±0.33	0.83±0.20	1.08±0.34
sterol esters/						
wax esters	---	---	---	---	---	---
methyl esters	---	---	---	---	---	---
triacylglycerols	---	3.08±0.47	2.55±0.36	4.08±0.36	2.40±0.16	0.66±0.66
free fatty acids	26.68±1.99	23.01±0.90	31.72±3.75	25.85±1.09	14.34±0.70	10.99±1.59
free aliphatic						
alcohol	---	---	---	---	---	---
free sterol	8.66±0.43	7.39±0.35	7.97±1.63	8.96±1.05	12.44±2.33	10.23±0.27
diglycerols	2.44±1.08	0.81±0.23	1.57±0.84	---	---	---
acetone-mobile						
polar lipids	8.34±0.39	8.64±0.38	10.42±0.98	7.91±0.41	8.58±0.48	8.70±0.54
phospholipids	52.36±1.95	55.81±0.12	45.28±2.76	51.33±1.76	61.42±2.07	68.36±1.80

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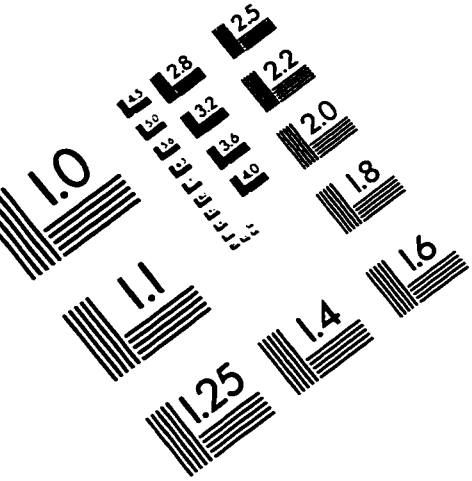
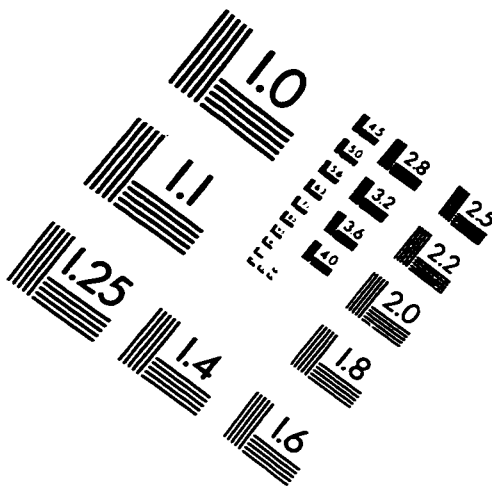
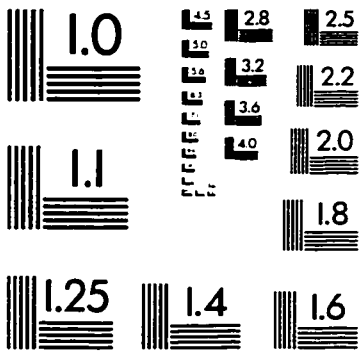
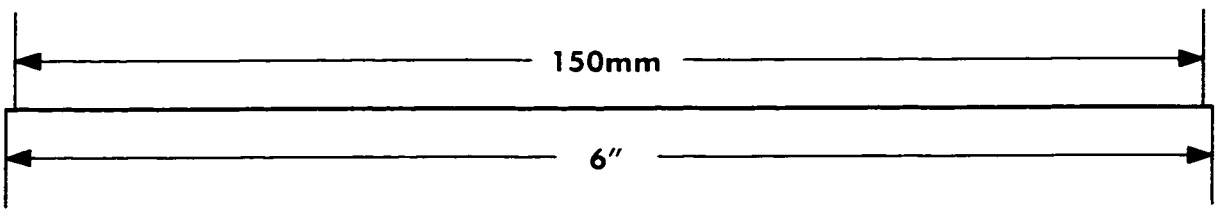
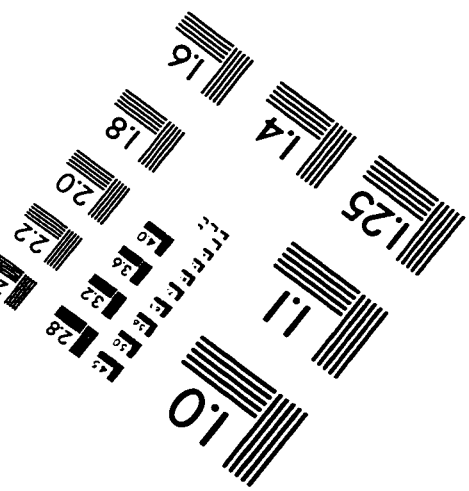
IMAGE EVALUATION TEST TARGET (QA-3)

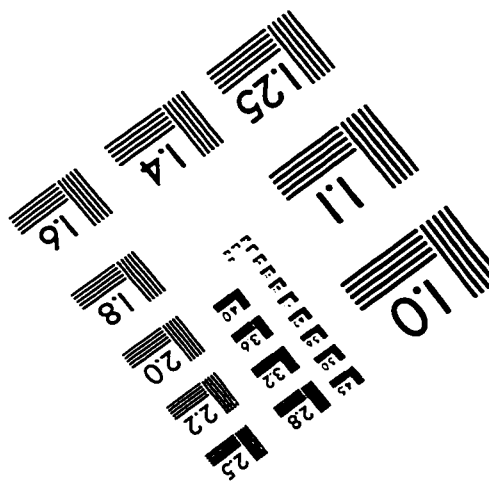












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